HUNTINGTON MEDICAL RESEARCH INSTITUTES

NEUROLOGICAL RESEARCH LABORATORY

734 Fairmount Avenue

Pasadena, California 91105

Contract No. NO1-NS-5-2333

QUARTERLY PROGRESS REPORT

October 1 - December 31, 1995

Report No. 1

"MICROSTIMULATION OF THE LUMBOSACRAL SPINAL CORD"

William F. Agnew, Ph.D.

Randy R. Carter, Ph.D.

Barbara Woodford, Ph.D.

Douglas B. McCreery, Ph.D.

Leo A. Bullara, B.A.

This QPR is being sent to you before it has been reviewed by the staff of the Neural Prosthesis Program

Abstract

In this quarter, we report on three chronic experiments aimed at examination of the effects of chronic electrical stimulation on spinal cord tissue. In two of these, current leaks developed in the electrode arrays, preventing stimulation. In the other experiment, the chronic stimulation protocol was completed but the histologic results were lost due to corruption by mislabeled chemicals.

We also report the results of four acute experiments. In one, administration of a striated muscle blocking agent had no effect on stimulation-induced urethral pressure decrease, while the administration of a smooth muscle blocking agent eliminated this response. In a second experiment, instability caused by respiration-induced movement of the spinal cord and displacement of the stimulating electrode prevented interpretation of the effects of the two drugs. In two other acute experiments, complete urethral pressure profiles were recorded using a new, smaller catheter. The affects of administering striated and smooth muscle blocking agents were recorded.

2

Introduction

The overall goals of this contract are to develop a method of chronic microstimulation of the sacral cord of the cat to effect micturition, and to evaluate the effects of the electrical stimulation on neural and surrounding tissues. The results from chronic experiments have been limited due to technical difficulties. Two of these were initiated in the last quarter and completed in this quarter and one was initiated in this quarter. All three experiments were intended to be studies of electrodes implanted for three weeks and then the electrodes were to be pulsed continuously for 8 hours on each of two successive days. The electrode assemblies developed leaks in two experiments and the histologic results were lost in a third experiment. The cause of the electrode assembly leakage has been traced to a change in fabrication technique and has been corrected. The histologic results were corrupted by the use of inappropriate chemicals as a result of mislabeled containers.

Four acute experiments have also been performed this quarter. These were designed to further examine the decrease in intraurethral pressure noted during stimulation in the spinal cord. In one experiment, SP-54, administration of pancuronium, which blocks the neuromuscular junction of striated muscle, did not significantly affect the stimulation-induced decrease in intraurethral pressure or increase in bladder pressure. Administration of hexamethonium, a smooth muscle blocker, eliminated the stimulation-induced pressure changes within the bladder and urethra. In a second experiment, SP-55, the response generated by stimulation in the spinal cord was not sufficiently stable to accurately judge the effects of the pharmacological agents. Steps have been taken to improve the mechanical stability of the spinal cord during these experiments with the goal of improving the stability of the elicited responses. In the remaining two experiments (SP-56 and SP-57), a solid-state pressure transducer was passed along the length of the

urethra and into the bladder to determine the pressure profiles during normal conditions and after administration of pancuronium and hexamethonium.

Methods

Chronic Experiments. Adult male cats were anesthetized with 50% nitrous oxide and 1-2% Halothane and the spinal cord exposed as described previously. The S_2 region of the spinal cord was localized by stimulation of the dermatome it serves while recording the dorsal cord potential supradurally, as described previously. A substrate to support the electrodes was fabricated from a silicone tube (508 μm id, 940 μm od, 6 mm long) that was halved lengthwise and either perforated with holes (203 μm dia) or transverse slits and was placed over the dorsum of the cord. Four activated iridium microelectrodes (50 μm dia., 2.8 mm long, 2000 μm^2 exposed stimulating surface) were implanted manually at approximately the dorsal midline of the spinal cord and angled outward at about 10 degrees. The electrodes were pulsed individually and the bladder lumen pressure and intraurethral tone were monitored. The electrodes were advanced into the cord until good elevation of bladder pressure was produced by the stimulation. The dura was then closed over the electrodes and the effect of the stimulation again measured. A silastic pad (to which the stimulating electrode leads had been glued) was sutured to the dura to reduce traction on the electrodes. The ground electrode was sutured in place over the microelectrodes. A small hole was made in the dura and a recording electrode inserted and threaded so as to lie approximately 3 cm caudal to the stimulating electrodes. A suture was used to secure the recording electrode to the dura. A reference electrode was sutured to the muscle 2 cm above the dura. The wound was flushed with antibacterial solution and the muscle and skin were closed in layers. Subsequent recordings were made with the animal anesthetized with Pentothal (i.v., as needed) or Nembutal (i.v., as needed). A sterile catheter and sterile saline were used during recording of the bladder lumenal pressure.

Acute Experiments. Four adult male cats were anesthetized with 50% nitrous oxide and 1-2% Halothane. In two cats, the spinal cord was exposed using a dorsal

laminectomy and the S_2 region localized in the manner described above. The spinal cord was covered with light mineral oil to prevent drying. The stimulating electrodes were inserted into the cord using a standard stereotaxic apparatus.

In two other cats, a small (2.5 F) pressure transducer (Millar Instruments) was passed along the length of the urethra and measurements taken at 0.5 cm increments. Several runs were completed to determine repeatability of the obtained pressure profiles and the changes induced by administration of pancuronium or hexamethonium as described above.

All four animal were euthanized at the termination of the experiment without regaining consciousness. In the latter two cats, distance measurements along the urethra recorded during the experiment were correlated with anatomical measurements taken at autopsy.

Histology. At the end of the experiment the animal was anesthetized with Nembutal and perfused through the aorta with saline followed by 2 L of 1/2 strength Karnovsky's fixative (2% paraformaldehyde and 2.5% glutaraldehyde) in 0.1 M sodium cacodylate buffer, pH 7.4. With the electrodes *in situ*, the complete cord and spinal roots were dissected out to precisely localize the microelectrodes. Two-mm-thick transverse sections containing the electrode tracks were dissected, processed and embedded in epoxy resin. One-μm thick sections were cut serially through the blocks and examined using light microscopy.

Results

The results from chronic experiments have been limited due to technical difficulties. All three experiments were intended to be studies of the effects of chronic stimulation. Three weeks after implantation, the electrode assembly of animal SP-51 developed leaks such that chronic stimulation was not possible. In a second animal, SP-52, the electrodes remained viable throughout the 3 week implant period, chronic stimulation was delivered, and the animal was perfused. However, due to a mislabeling of chemical containers, histologic results were lost. In a third chronic experiment, SP-56, no response was achieved with spinal cord stimulation during surgery and, after closing the dura, the electrode assemblies showed indications of current leakage. The cause of this has been traced to a change in fabrication technique and has been corrected.

In an acute experiment, SP-54, our intent was to determine the effect of pharmacological agents on the previously described decreases in intraurethral pressure elicited by stimulation of the sacral spinal cord. Figure 1 shows several repeated periods of stimulation delivered over approximately 12 minutes. In all cases the stimulation consisted of 120 μA and 400 μs pulses delivered at 50 Hz with a duty cycle of 1 s on and 2 s off. A typical response to the stimulation can be seen in which the bladder pressure increases and the intraurethral pressure initially increases and then decreases substantially to well below baseline pressure. At the termination of the stimulation, the intraurethral pressure returned to baseline. Administration of pancuronium bromide (0.2 mg/kg, iv), a striated muscle blocker, did not affect the stimulation-induced pressure changes of either the bladder or urethra. Pancuronium has a rapid onset of effect (30 s) and a peak effect at 3 min. Figure 2 demonstrates this on a fast time base where the urethral pressure responses obtained before and after administration of the drug are shown superimposed.

In the same animal, hexamethonium bromide (2 mg/min, 12 mg total, iv), a smooth muscle blocker, was delivered with the aid of an infusion pump. Figure 3 shows two

stimulation periods (upper trace shows intraurethral perfusion pressure and lower trace shows bladder hydrostatic pressure), one before the drug was administered and one after, demonstrating that a smooth muscle blocking agent eliminated the response of both the bladder and urethra. This is demonstrated on a fast time base in Figure 4 where the urethral pressure responses obtained before and after administration of the drug are shown superimposed.

In a second acute experiment, SP-55, the responses of the bladder and urethra were not sufficiently stable to allow determination of the effects of the two drugs. It is believed that the instability stemmed from movement of the spinal cord relative to the electrode as a result of the animal's respiration. Steps have been taken to modify our spinal frame to eliminate this problem.

Urethral pressure profiles varied somewhat with time, as might be expected, although the qualitative shape of the responses was quite repeatable. Figure 5 shows the pressure measurements taken during two successive runs along the length of the urethra. These pressure recordings indicate hydrostatic pressure rather than perfusion pressure as in earlier experiments. In this experiment the bladder was infused with saline until full, as judged by palpation through the abdominal wall. Measurements were taken with the catheter inserted fully and then withdrawn slowly in 0.5 cm increments. After each movement, a pause of 10-20 s was allowed to minimize stimulation of receptors in the urethral wall. A scaled drawing based on measurements taken at autopsy is shown below the pressure profile in Figure 5 (IC/BC = ishiocavernosus and bulbocavernosus muscles, EUS = external urethral sphincter, PG = prostate gland). We feel that the pressure measurements taken distal to the IC/BC include significant force artifacts that can be attributed to the narrowing of the diameter of the urethra within the penis, especially near the tip.

The urethral pressure profile was significantly affected by a neuromuscular junction blocking agent. In Figure 6 (abbreviations are as in Fig. 5), this same preparation is shown before and after administration of pancuronium. Note the decrease in intraurethral pressure at the level of the EUS and more proximal and the slight increase in intraurethral pressure distal to the EUS.

In another animal, two urethral pressure profiles were recorded with the bladder infused with saline until only partially full as judged by external palpation (Figure 7, abbreviations are as in Fig. 5). Note, first, that the pressure recorded above the level of the prostate gland is quite low and, second, that the pressure at the level of the EUS is also low compared to that measured with the bladder filled completely. Administration of the smooth muscle blocker hexamethonium did not alter the pressure profile significantly.

Discussion

The results of the chronic experiments this quarter are disappointing but we feel that we have identified the causes and have instigated changes that we hope will prevent this from corrupting future experiments.

The results of the successful acute experiment are encouraging. The stimulation-induced decrease in intraurethral pressure appears to be mediated by smooth muscle. The striated muscle blocking agent, pancuronium, did not eliminate the decrease in urethral pressure, indicating that the external urethral sphincter and pelvic musculature may not play a significant role in this response. The smooth muscle blocking agent, hexamethonium, eliminated the intraurethral pressure decrease and, therefore, such a decrease must be caused by contraction of the smooth muscle as a result of stimulation. In other words, this suggests that stimulation of the spinal cord is not eliciting its effect through the activation of inhibitory interneurons that cause subsequent relaxation of the smooth muscle. Contraction of longitudinally arranged smooth muscle could cause a lowering of intraurethral pressure by stiffening and shortening the urethra, thus expanding its lumen.

By using a new, smaller, solid-state pressure transducer, it was possible to record the complete intraurethral pressure profile. In two experiments, we found that the profiles were qualitatively repeatable although quantitative differences were noted between successive runs. The repeatability is sufficient to note the affects of a drug administered to block the neuromuscular junction of striated muscle.

It is interesting that blocking the smooth muscle of the urethra and bladder did not cause significant changes in the urethral pressure profile. As noted above, our data suggests that the stimulation-elicited decrease in intraurethral pressure was not caused by relaxation of the smooth muscle but, rather, by its contraction. In that experiment (SP-54), blocking the smooth muscle eliminated contraction of the bladder during stimulation

and the concurrent decrease in the intraurethral pressure but did not eliminate intraurethral tone. While this second experiment (SP-56) did not involve stimulation, it supports the same argument since blocking the smooth muscle did not cause a significant decrease in intraurethral pressure near the level of the EUS.

Future work

In the next quarter we plan chronic studies aimed at evaluating the response of the spinal cord to chronic stimulation. Additional acute experiments will be conducted to further examine the effects of muscle blocking agents on the bladder and intraurethral pressure changes evoked by spinal stimulation. We also plan to begin testing a new design for the intraspinal electrode arrays (Intraspinal Type II).

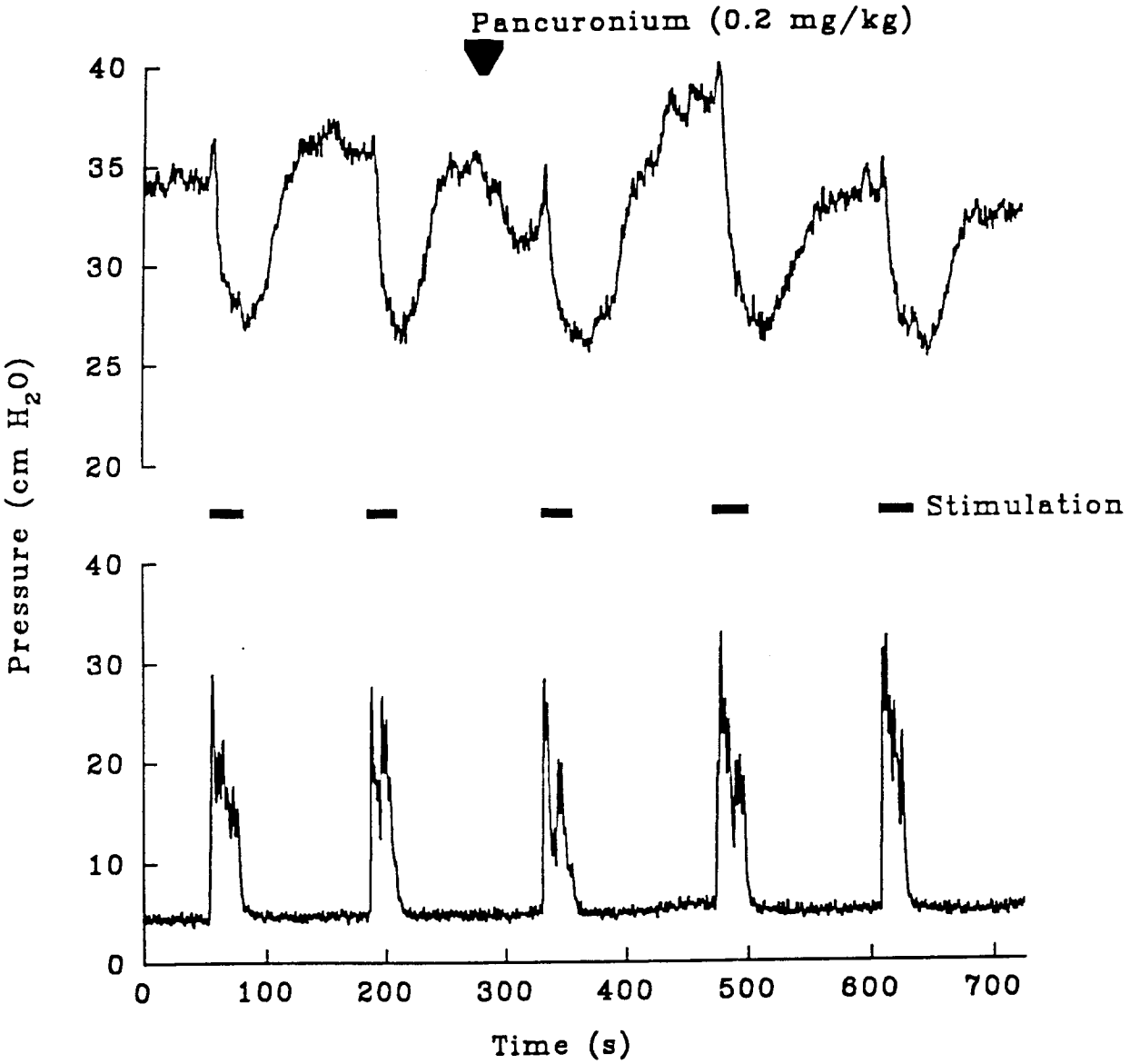
Pancuronium (0.2 mg/kg)
40
35
30
25
20
Stimulation
40
30
20
10
0
Pressure (cm H_2O)
0
100
200
300
400
500
600
700
Time (s)

Figure 1.

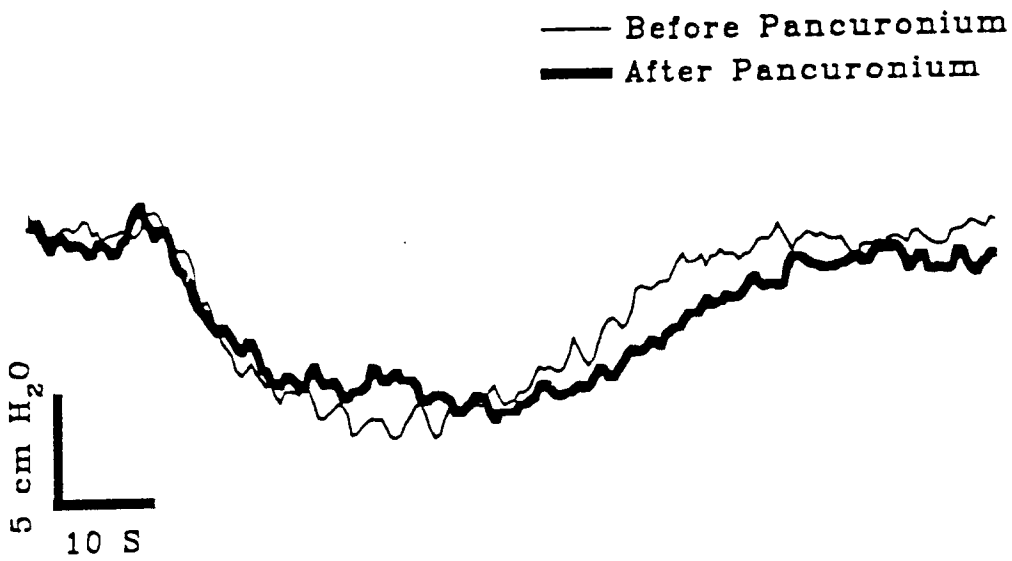

Figure 2.

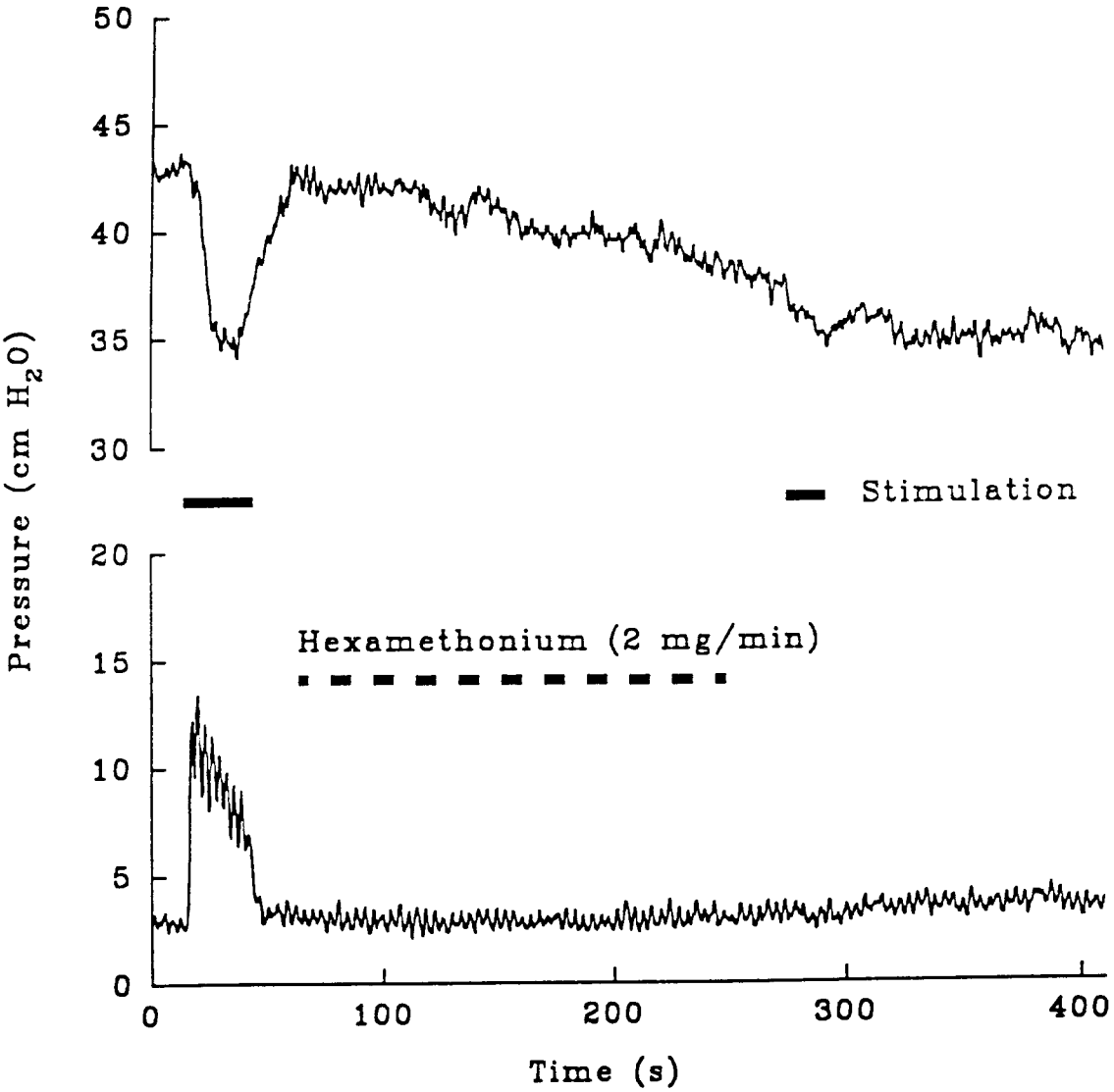
50
45
40
35
30
Pressure (cm H_2O)
Stimulation
20
15
10
5
0
Hexamethonium (2 mg/min)
0
100
200
300
400
Time (s)

Figure 3.

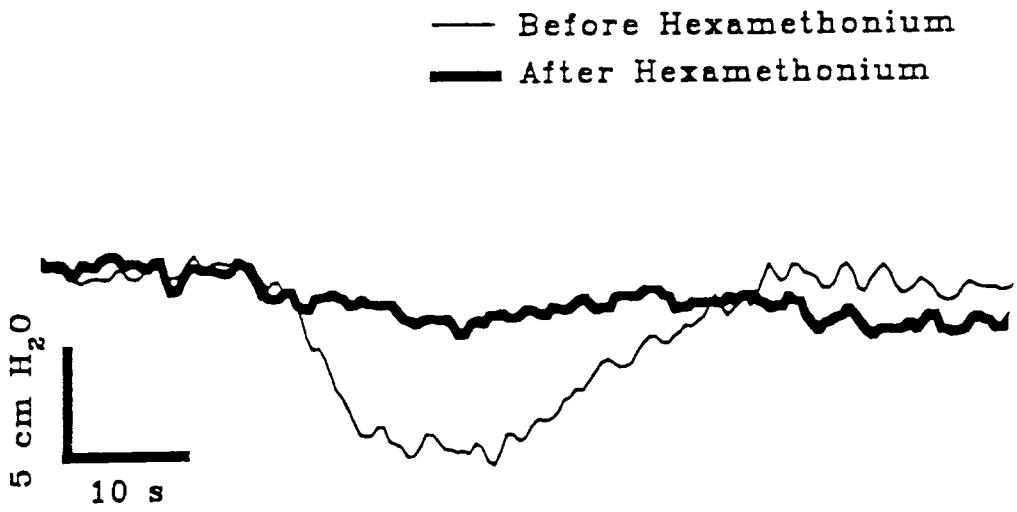

Figure 4.

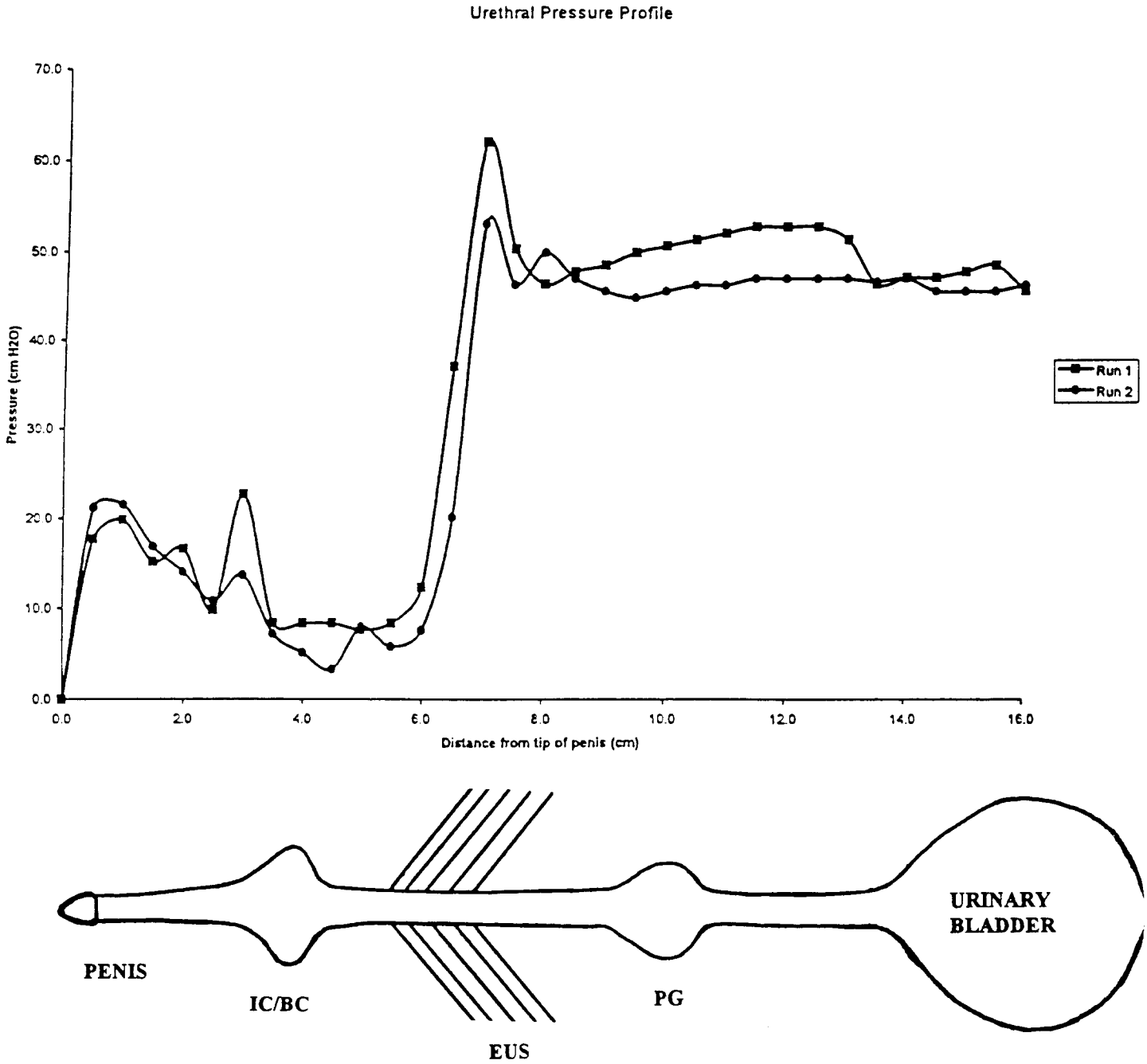
Urethral Pressure Profile
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Pressure (cm H2O)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
Distance from tip of penis (cm)
Run 1
Run 2
URINARY BLADDER
PENIS
IC/BC
PG
EUS

Figure 5.

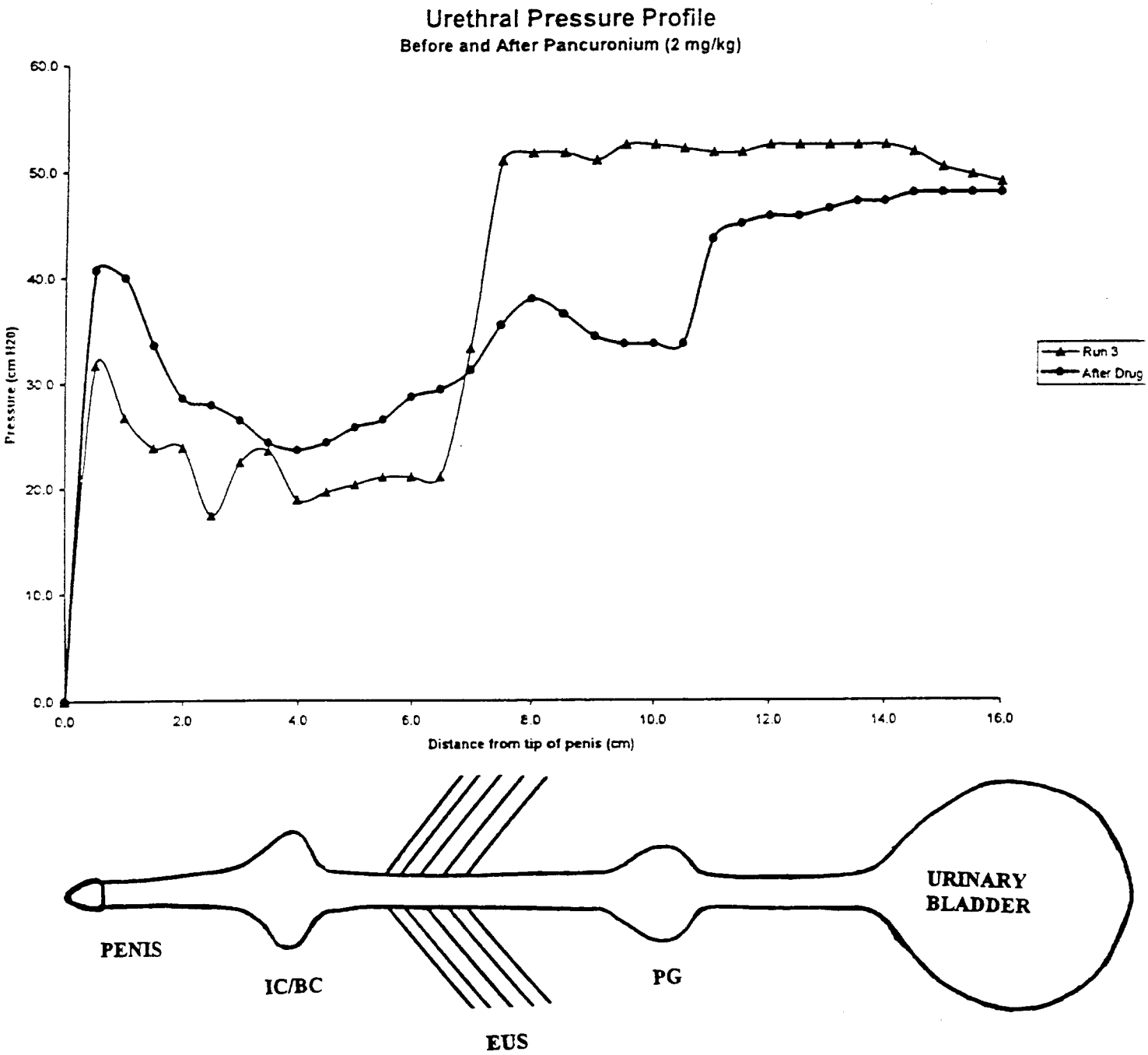
Urethral Pressure Profile
Before and After Pancuronium (2 mg/kg)
Pressure (cm H20)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
Distance from tip of penis (cm)
Run 3
After Drug
URINARY BLADDER
PENIS
IC/BC
PG
EUS

Figure 6.

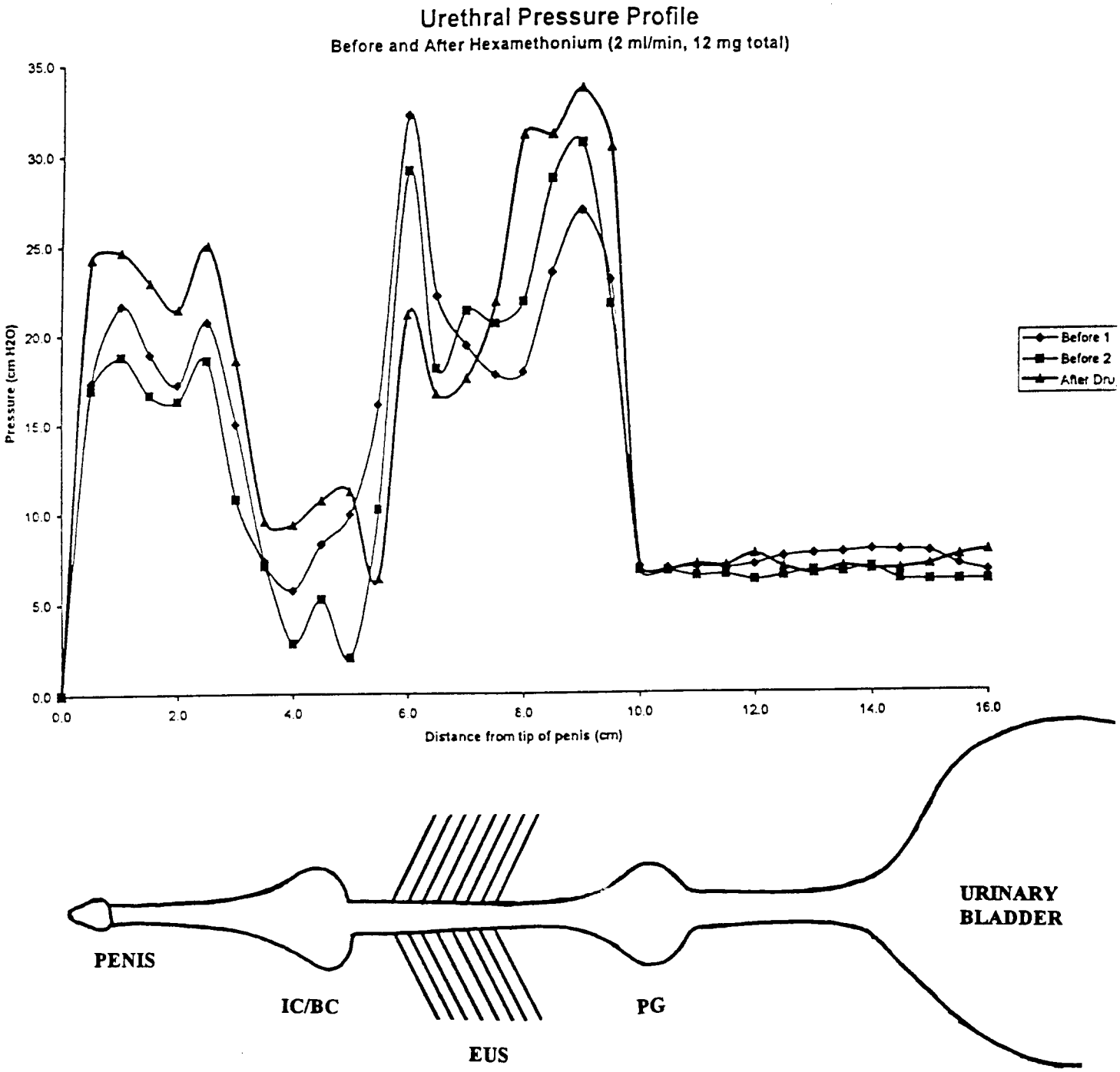
Urethral Pressure Profile
Before and After Hexamethonium (2 ml/min, 12 mg total)
Pressure (cm H2O)
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
Distance from tip of penis (cm)
Before 1
Before 2
After Dru
URINARY BLADDER
PENIS
IC/BC
PG
EUS

Figure 7.